STUDIES ON INTERACTION OF ROOT-KNOT NEMATODE AND RHIZOSPHERE FUNGI IN TOMATO

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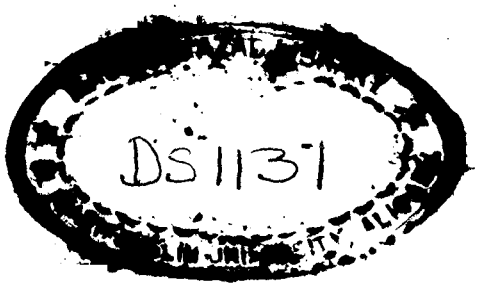

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INTRODUCTION

Chapter - I

INTRODUCTION

Vegetables constitute an important part of the diet of human being and specially in India where vast majority of population is vegetarian or thrives on vegetables. They are source of proteins, vitamins and minerals (Work and Carew, 1955). The yield of different vegetables in India is far below the yield in developed countries. One of the important reasons of the low yield is the damage caused by the diseases including nematode (Webster, 1972). Amongst different vegetables, tomato (Lycopersicum esculentum) is of no less importance and is grown throughout India. The crop suffers due to ravages of diseases such as Damping off of seedlings by Pythium aphanidermatum, P. indicum, Phytophthora parasitica; Fusarium wilt and root rot by Rhizoctonia solani; leaf spot by Cercospora feligera; leaf mould Cladosporium fulvum; blight Phytophthora infestans; black eye rot, P. parasitica and soft rot Oospora lactus parasitica.

Among nematodes, root-knot nematode is of great importance, It has been reported to cause losses upto the extent of 50-80% in case inoculum rate is high (Van Berkum and Sheshadri, 1970). In the presence of certain soil borne fungal pathogens the losses due to nematode are further compounded (Webster, 1972). These interaction between nematode and fungi are of various types such as

plant nematodes breaking the resistance of cultivar against fungal diseases (Jenkins and Coursen, 1957; Davis and Jenkins, 1963; Bowman and Bloom, 1966), synergistic or additive (Palmer and McDonald, 1974; Garcia and Mitchell, 1975). There is immense amount of literature on the interaction of nematodes with soil borne pathogenic fungi which has been reviewed from time to time by Powell (1963), Pitcher (1965), Miller (1965), Weischer (1968), Bergeson (1972).

The interesting aspect of nematode fungus interaction is that sometimes in the presence of nematodes even a non pathogenic fungus becomes pathogenic and a weak pathogen causes severe damages to the crops (Powell, 1971).

Studies on disease complexes have mostly been done under conditions where only two pathogens were present. But in nature this situation seldom exists. Other micro-organisms present in rhizosphere of plants might be affecting the nematode and the fungal pathogens involved in disease complexes. But this kind of information is completely lacking. Moreover tomato is a transplanted crop, where seedlings are grown in nurseries and planted elsewhere. Therefore the chances are that seedlings are exposed to nematodes and other pathogens both at the nursery and fields of transplantations. The information on disease complex development in such a situation is also meagre. Therefore, keeping in view of these facts, it is

considered desirable to study the following:-

1) Rhizosphere and rhizoplane mycoflora of tomato in the nursery and in the fields where the transplantation is done at peak periods of growth;

2) Effect of certain rhizosphere non pathogenic fungi on germination of seeds of tomato in the presence of nematode and pathogenic fungi;

3) Effect of inoculating the seedlings of tomato raised in presence of nematode and pathogenic fungi with non pathogenic and pathogenic in the presence of root-knot nematode;

In these studies the effect of rhizosphere fungi on disease complexes will be determined seperately and in different combinations;

4) Effect of culture filtrate of different rhizosphere fungi on hatching and mortality of larvae of root-knot nematode and working out LD 50;

5) Effect of different carbon and nitrogen sources on growth of the fungus showing highest antagonistic effect with a view to find out the factors affecting maximum production to toxic metabolites.

REVIEW OF LITERATURE

Chapter - II

REVIEW OF LITERATURE

NEMATODE FUNGUS INTERACTIONS

Plants are always exposed to a number of pathogenic organisms occurring in their surroundings and thus in nature disease development is never an outcome of the activity of one pathogen, but total sum of all the factors including other pathogens coexisisting and the environment. All these pathogenic microorganisms act seperately and interact with others. The damage done by such interactions of pathogens is often compounded (Powell, 1971).

Plant parasitic nematodes occupy the same niche in the soil as other pathogens do i.e. in the rhizosphere of roots. During their existence there, these nematodes interact with other pathogenic microbes such as fungi, bacteria, nematodes and viruses.

The literature on the aspects of interaction of nematodes with other microorganisms has been reviewed by Pitcher (1965), Powell (1971) and Weischer (1968). Pitcher (1965) suggested that interactions between nematodes and other microorganisms can be grouped as follows -

1) Nematode may act as vector of pathogen incapable of self establishment unless introduced below the host epidermis.

2) Acts as vector of pathogen capable of self establishment once in contact with host.

3) Provide necrotic infection court for certain types of fungi and help them in their establishment.

4) Provide mechanical portals or acts as mechanical wound agents.

5) Acts as breaker of disease resistance of crops.

6) Act as modifires of substrate for colonization of other pathogens.

7) Acts also as deterrants.

Interaction of plant parasitic nematodes have been reported with viruses, bacteria (including root nodule bacteria) fungi, mycorrhiza and other pathogenic and non pathogenic nematodes.

According to Powell (1971) the various interactions between nematode and fungus have been categoriesed as under-

a. Nematode - Fungus wilt disease interactions
b. Nematode - Fungus root rot disease interactions.
c. Nematode - Fungus seedling disease interactions.

a) <u>Nematode Fungus wilt disease interactions</u>

Amongst various soil borne pathogens, wilt causing organisms are serious pathogens of crops of economic importance and in the presence of nematodes the losses caused by them are greatly increased.

The first report of interaction of wilt causing organisms and the nematode was that of Atkinson (1892) who observed that root-knot nematode increased Fusarium wilt in cotton. Since then large number of incidences of interaction of nematode and wilt causing agencies have been reported. In most of the cases the reports indicate an increase in the severity of wilt due to the presence of nematode exhibiting a direct co-relation between the degree of wilt development and the population of nematodes.

Martin et al (1956) observed that among the five nematodes tested by them (Meloidogyne incognita, Meloidogyne incognita acrita, Trichodorus, Tylenchorhynchus, Helicotylenchus), M. incognita and M. incognita acrita considerably increased the severity of Fusarium wilt. Jenkins and Coursen (1957) reported that due to the presence of root knot nematode in association with wilt causing organism, the wilt resistant variety become susceptible. Thus there was a break in the resistance due to the presence of the nematode. Davis and Jenkins (1963) also reported the break in resistance of pea variety 'Alaska' to F. oxysporum f. pisi race-1 in presence of M. incognita. Bowman and Bloom (1966) observed an indirect relationship of M. incognita to the breaking of resistance of cultivars to Fusarium wilt. They reported that in fact M. incognita changed the physiology of host plant. Summer and Johnson (1973) reported that more plants of watermelon were wilted due to F. oxysporum f. niveum when M. incognita was present and symptoms were

more prominent in resistant cultivar than in susceptible ones. The severity of disease increased with increase in inoculum densities of nematode. Holdeman and Graham (1954) reported that Belonolaimus gracilis broke the resistance of cotton cultivar resistant to Fusarium wilt. Similar results were obtained by Binder and Hutchinson (1959). Thomason (1958), while working with black eye wilt of bean caused by F. oxysporum f. tracheiphillum (E.F.Sin) Snyder and Hansen, noticed that variety "Chino 3" exhibited greater severity in the presence of M. javanica. Moreover the resistance of var 'Grant' was broken when the nematode was present.

Schinder et al (1959) pointed out that while endo parasites M. incognita, M. arenaria thamesi, M. incognita acrita and M. javanica increased the incidence of carnation wilt caused by F. oxysporum f. dianthii (Prill and Del) Snyder and Hansen, no significant effect was observed in the presence of ectoparasites like Helicotylenchus nannus, Rotylenchus buxophilus. Perry (1961,63) obtained similar results on the interaction of F. oxysporum f. vasinfectum and M. incognita on cotton. On the other hand, Giamalva et al (1962) did not get any significant effect of root knot nematode on Fusarium wilt in sweet potato caused by F. oxysporum f. batatus Snyder and Hansen. Minton and Minton (1963), while studying the effect of root tissue galled by M. incognita acrita on growth of F. oxysporum f. vasinfectum in cotton, observed that the

fungus grew well in nematode infected tissue, while poorly in healthy tissue of plants. Melendez and Powell (1965) determining the histological and pathological effects of root knot nematode on Fusarium wilt of flue cured tobacco also noted ready penetration and extension development of fungal hyphae in giant cells. Agrawal et al (1974) observed that the extract of ginger infected with root knot nematode stimulated the linear growth and hyphal thickness of Fusarium oxysporum f. zingiberi. Cooper and Brodie (1963) observed that root knot nematode is a higher predisposing agent than sting nematode. Ross (1962) found that cyst nematode (Heterodera glycines) was more effective than root knot nematode in predisposing the soybean plants to Fusarium wilt. Sikora (1977) also found greater damage to clover when F. avenaceum and F. oxysporum were consistently associated with Heterodera trifolii, but Muller (1975) found reduction in growth of tomato by Fusarium wilt in presence of H. tabacum.

The sequence of presence of the two microorganisms also influenced the interaction between them. Porter and Powell (1967) found that the quantum of damage to tobacco seedlings was less in the event of simultaneous inoculation of plants with F. oxysporum f. nicotianae and M. incognita or M. arenaria or M. javanica than to those in which nematode inoculations preceded the fungal inoculation by 2-4 weeks. Goode and McGuire (1967) pointed out that nematode predisposed tomatoes to avirulent races of F. oxysporum f. lycopersici. They also suggested that probably the fungus

mutated within the host. Powell and Batten (1969) observed that M. incognita played an important role for invasion of secondary organisms like Alternaria tenuis. Johnson and Litterell (1969) reported that the presence of M. javanica greatly enhanced the severity of Fusarium wilt, however, the wilt severity was less with M. incognita or M. hapla.

Additive interaction has also been reported on okra with M. incognita and F. oxysporum f. lycopersici together (Khan and Saxena, 1969). Hirano and Kawamura (1972) pointed out that in the presence of larvae of M. incognita acrita, roots of non host plants were penetrated by F.oxysporum f. lycopersici and F. oxysporum f. niveum (E.F.Sin) Snyder and Honsen. Liburd and Mai (1976) reported that F. oxysporum f. lycopersici and M. incognita together not only resulted in earlier appearance of wilt symptoms but also in increased incidence of disease, Carter et al (1977) pointed out a reduction of 9, 16 and 47% in dry weight of shoot of susceptible cultivar of tomato when inoculated with M. incognita and F. oxysporum f. lycopersici alone and nematode with fungus respectively. However, no such reduction in dry weight was reported in resistant cultivar.

An antagonistic interaction has been reported between F. oxysporum and F. solani with M. incognita and a synergistic reaction between F. graminaceum and F. equisetii with M. incognita in soybean (Goswami and Agrawal, 1978). Nematode infected plants were found to

be more damaged by fungi than non infected ones. Griffin and Thyr (1978) observed that when 14 days old seedlings of lucerne plants were inoculated with M. hapla followed by inoculation with F. oxysporum after thirty days, there was significant reduction in growth of plants of susceptible variety and not that of resistant cultivars. Garber et al (1979) studied interaction in terms of population levels of F. oxysporum f. vasinfectum and M. incognita on cotton and reported that infection with nematode increased the probability of fungal infections, which was however, not severe in the absence of nematode.

Lopes and Lordello (1979) reported that association of both M. incognita and F. solani f. piperi was more harmful than either of the pathogen alone. In an attempt to control the disease complex Aboul Eid et al (1981) studied effect of fensulfothion on root knot nematode and Fusarium wilt and other microorganisms in the rhizosphere of tomato. The nematicide adversely affected the infection caused either by F. oxysporum or M. incognita but increased the number of spore former actinomycetes in rhizosphere. Kareev et al (1983) reported that the presence of nematicide made a way for early appearance of wilt symptoms on susceptible cotton plants.

Neal (1954) reported a direct relationship between the degree of occurrence of Fusarium wilt and presence of reniform nematode (Rotylenchulus reniformis).

O' Bannon *et al* (1967) observed an interaction of *Tylenchulus semipenetrans* and *F. solani* and *F. oxysporum* at different temperatures. They observed no reduction in growth of plants inoculated with *F. oxysporum* but those inoculated with *F. solani* indicated greater reduction in growth. However, at 30°C synergistic interaction was noticed between *F. solani* and nematode, while at 20-25°C it was only additive.

Nematodes other than sedentry endoparasites are also capable of forming disease complexes with *Fusarium*. Newhall (1958) observed that *Fusarium* wilt in banana caused by *F. oxysporum* f.*cubens* (E.F.Sin) Snyder and Hansen, was increased two times in presence of *Radopholus similis* in soil.

Seinhorst and Kunijasu (1971) also reported that wilting of peas var. 'Rando' caused by *F. oxysporum* f. *pisi* race-2 was enhanced by the presence of *Pratylenchus brachyurus* more on wilt susceptible varieties than on resistant ones; and the percentage of wilting was higher in plants inoculated with nematode and fungus simultaneously than those inoculated with nematode prior to fungus. Bee Rodriguez and Ayala (1977) working on interaction of *Pratylenchus zeae* with *Curvularia* sp, *Fusarium moniliforme*, *Rhizoctonia solani* and *Macrophomina* sp. on sorghum found that all fungus + nematode combinations caused reduction in growth, however this reduction was highest in combination of *P. zeae* with *Curvularia* sp.

Labruyere et al. (1959) observed considerable increase in the severity of yellowing caused by F. oxysporum f. pisi race-3 on peas. Similarly Shanmugum et al (1978) found increased Fusarium and Verticillium wilt on cotton in the presence of Hoplolaimus.

Cooper and Brodie (1963) reported an increase in severity of Fusarium wilt on cotton in presence of stin nematode (Belonolaimus gracilis).

Verticillium is another wilt fungus,causing seve: significant disease complexes with plant parasitic nematodes. Root lesion nematode has been found to be t most popular nematode in forming the disease complexes with Verticillium. Mckeen and Mountain (1960,62) repor a synergistic interaction of Pratylenchus penetrans and Verticillium albo atrum on tomato, while Bergeson (1963 observed an additive interaction between the above two on peppermint; Faulkner and Skotland (1965) on eggplant McDonald (1967), Morsink and Rich (1968) on potato; Cooseman (1979) on strawberry and Mckeen and Talboys (1 on chrysanthemum. Faulkner and Bolander (1969), while studying the interaction of Pratylenchus minyus and Verticillium dahliae on peppermint at different soil temperatures, observed that nematode increased both the incidence and severity of wilt on host plant at 24°C. Conroy et al (1972) reported that P. penetrans consistently increased the infection of disease caused by V. albo-atrum on tomato roots at different inoculum

densities of Verticillium. Infection of potato root by P. penetrans and V. albo-atrum has been found to result in decreased incubation period for fungus infection (Burpee and Bloom, 1974). Amongst five species of Pratylenchus only P. penetrans and P. vulnus could induce the symptoms of wilt in Impatiens balsamina (Muller,1977). Cooseman (1979) noticed a synergistic effect on flax (Linum usitatissimum) when Verticillium spp. and P.penetrans (Cobb, 1917) were present together. Sudakova (1981) reported an increase in Verticillium attacked plants by 50% and 45% in presence of Aphelenchus avenae and Pratylenchus brachyurus respectively.

Verticillium has also been found to form effective disease complexes with other plant parasitic nematodes. Verticillium wilt development on tobacco was more severe in presence of Heterodera tabacum (Miller, 1975), and on potato in the presence of root knot nematode (Jacobson et al 1979). Nematode populations were higher in plants infected with the fungus and nematode together than on the plants infected with nematode alone at 24°C, however, there was no difference at 30°C.

An additive effect in the reduction of the growth of cotton was observed when plants were inoculated with Verticillium dahliae and Rotylenchulus reniformis. In these studies rhizosphere microorganisms were found to play a role in increasing the degree of damage caused by both the organisms (Tchatchoua and Sikora, 1978).

Black shanck of tobacco caused by Phytophthora parasitica var. nicotianae causes a considerable amount of root decay. The fungus is very aggressive pathogen in this host and causes rapid death in susceptible individuals. The fungus forms important disease complexes with root knot nematode where increased severity in the incidence is observed (Sasser et al 1953, 1958, Powell and Nusbaum, 1960). Sasser et al (1953,55) and Miller (1968) reported that Meloidogyne along with Phytophthora parasitica var. nicotianae broke the resistance in black shanck resistant varieties.

Root lesion nematode (Pratylenchus spp) also increased the rapidity of development of black shanck and severity of disease, although this nematode remained unable to break the resistance of black shanck fungus resistant varieties (Inagaki and Powell, 1979). On the other hand, McIntyre et al (1978) reported that infection by lesion nematode protected the variety WS 117 against black shanck fungus. In a survey of rhizosphere of soybean Fusarium, Rhizoctonia solani and Pythium spp. were found to be commonest among pathogenic fungi, while Endogone calospore was most prevalent among endomycorrhizal fungi. Incidence of Rhizoctonia was increased by the soybean cyst nematode and incidence of Pythium spp. increased and endomycorrhyzal fungi decreased by root knot nematode (Shenck and Kinolch 1974).

Hussey and Roncadori (1978) observed that Pratylenchus brachyurus with endomycorrhizal fungus Gigaspora margarita did not reduce the growth of Gossypium hirsutum, however, the mycorrhizal activity in cotton roots suppressed the number of nematodes in the host.

The literature on role of mycorrhizal fungi on the nematode control has been reviewed by Shenck (1981). Casson et al (1983) studied the interaction of vesicular arbuscular mycorrhizal fungi (Gigaspora margarita or Glomus mossae) with M. incognita on tomato and found that inoculation of mycorrhizal fungi did not alter the infection by the nematode regardless of phosphorus level.

O' Bannon et al (1980) pointed out that infection site of citrus nematode (Tylenchulus semipenetrans) in roots grown in mycorrhizae infested soil exhibited arbuscules development but not the vesicles. O' Bannon et al (1980) observed a favourable response of citrus lemon seedlings to a symbiont Glomus etunicatus in the presence of Radopholus similis.

Pratylenchus minyus and Rhizoctonia solani together caused considerable reduction in the growth of winter wheat as compared to that caused by either of the pathogen alone (Mountain and Benedict, 1956); whereas damage caused by R. solani to soybean was merely additive in presence

of nematodes. Helerodera glycines, Pratylenchus scribneri and Tylenchorhynchus martini, however, R. solani inhibited nematode population and development of H. glycines but its effect on F. martini was not very pronounced (Dave, 1977).

Cook (1970) while studying interrelation of cereal cyst nematode (Heterodera avenae) and take-all fungus (Ophiobolus graminis) on barley found that low levels of nematode appeared to be associated with high levels of fungus in the development of disease complexes.

Nath et al (1977) observed suppression in germination of seeds of eggplants when the seeds were grown in soil infected with Meloidogyne incognita and Ozonium texanum var. parasiticum simultaneously.

Meloidogyne incognita and Uromyces phaseoli have been found to reduce the fresh weight of beans to a greater extent when present in the same plant as compared to infection with either of the pathogen alone (Bookbinder and Bloom 1977).

Khan et al (1981) found that M. incognita and Phompsis vexans in combination reduced the growth of eggplant.

b) **Nematode-fungus root rot disease inferactions**

Several nematode genera played an essential role in development of root rot diseases caused by different

fungi. These type of disease complexes proved more harmful to plants than wilt diseases. Several instances have been reported in which nematode and fungi causing root rot when present together caused much more damage (Powell, 1971).

The incidence of root rot caused by Fusarium on lemon was more when associated with Tylenchulus semipenetrans (O'Bannon et al 1967) and to corn when associated with Pratylenchus scribneri (Palmer et al 1967) and Tylenchus agricola (Kiesel et al 1969). Gill and Swaroop (1977) reported that Heterodera avenae with F. moniliforme or Helminthosporium gramineum formed negative co-relations on growth of barley plants but when both the nematodes were present together, nematode multiplication was significantly increased. Sikora (1977) observed an increase in root rot of Trifolium subterraneum caused by Fusarium sp in presence of Heterodera trifolii Palmer (1969) pointed out that maize hybrid having highest susceptibility of M. incognita also had the highest incidence of root rot with Fusarium. More or less a synergistic interaction has been reported between Fusarium moniliforme and Meloidogyne incognita (Palmer and McDonald, 1974). An antagonistic interaction of Heterodera schachtii and Fusarium oxysporum on sugar beet has been reported by Jorgenson, 1970.

Mayol and Bergeson (1969,70) studied the secondary microbial invasion of tomato plants already inoculated with

M. incognita under septic and aseptic condition and found that reduction in growth of plants was more in septic condition in comparison to aseptic conditions.

Pythium is one of the important genus among the fungi causing root rot diseases and has been found to form disease complexes with several plants.

Apt and Koike (1962) observed that Meloidogyne incognita acrita and Pythium graminicola showed a positive interaction on top growth but not on shoot growth of sugarcane plants. Litterell and Johnson (1969) reported that Iceberg chrysanthemum plants showed severe symptoms when inoculated with Pythium aphanidermatum and Belonolaimus longicaudatus or M. incognita, however, the effect was more adverse with the former nematode. Melendez & Powell (1969) found that resistance of tomato variety NC-95 was broken when plants were inoculated with Meloidogyne incognita preceded by the fungus Pythium ultimum or Trichoderma harzianum by four weeks. Powell et al (1971) studied the disease complexes formed by M. incognita and either Botrytis, Pythium, Aspergillus, Penicillium, Curvularia or Trichoderma on tobacco and observed that highest necrosis occurred in treatments where nematode preceded any one of the fungus by several weeks.

Predisposition of roots of flue cured tobacco to P. ultimum was observed in presence of M. incognita (Melendez and Powell, 1970). Litterell and Johnson (1970)

reported that Belonolaimus longicaudatus, M. incognita and Pythium aphanidermatum when combined together significantly suppressed multiplication of both the nematodes and caused considerable reduction in plant growth. A synergistic interaction has been reported between Pythium myriotylum and/or Fusarium solani and Meloidogyne incognita on peanut (Garcia and Mitchell,1973) while Santo and Holtzman (1970) reported an additive interaction on sugarcane between Pratylenchus zeae and Pythium graminicola. Similarly a synergistic interaction between Pythium irregulare and Criconemoides quadricornis and F. solani and C. quadricornis on pecan roots has been reported by Hsu et al (1973).

Wyllie and Taylor (1960) observed increase in severity of root rot of soybeans by R. solani in presence of M. hapla. Irvine (1965) found highest killing of alfalfa plants infected with M. hapla and R. solani at 20-30°C, there was no effect at 15°C, even when the plants were inoculated with both the pathogens. Mature plants of tobacco were more damaged when inoculation with R. solani was followed by M. incognita but the nematode was unable to break the resistance of the cultivar (Powell and Batten, 1967). Golden and Van Gundy (1972) stated that prior infection by M. incognita changed the permeability of root cells by increasing the electrolyte leakage and thus making the plant susceptible to R. solani and Thielaviopsis basicola. Golden and Van Gundy (1975) found

that infection of M. incognita permitted extensive invasion of R. solani in galled roots. Chhabra et al (1977) reported that simultaneous inoculation of M. incognita and R. solani caused greatest damage to okra plants Azam et al (1979, 1980) found that extract of roots of brinjal plants infected with M. incognita stimulated the growth of R. solani, Pythium and Colletotrichum atramentarium. While culture filtrate of above mentioned fungi reduced the hatching of M. incognita larvae.

Benedict and Mountain (1954) observed synergism between R. solani and Pratylenchus minyus in winter wheat.

Dunn and Hughes (1964) reported that the growth of tomato was reduced more when Globodera rostochiensis entered the roots prior to R. solani and Colletotrichum atramentarium (Berk & Br) Taub than when the nematode preceded by the fungus or when the entrance of two pathogens was simultaneous.

Patel et al (1984) while working out the interaction between three nematodes spp. and Gauemannomyces graminis var. tritici and R. solani found that the disease was controlled to some extent by Aphelenchus avenae.

Haglund and King (1961) reported an increase in the severity of root rot of peas caused by Aphenomyces euteichus in presence of nematode Tylenchorhynchus martini. The increase of severity, however, was related to population of nematode directly. Minton and Jackson (1967) stated that

the presence of M. incognita and M. hapla speeded up the attack of Aspergillus flavus Link on peas,while Jackson and Minton (1968) found no effect of Pratylenchus on pod rot of peanut caused by A. flavus or A. niger van Tieghm.

Goswami et al (1970) reported that eggplant seedlings were damaged to the extent of 2.5% when they were inoculated with M. incognita and Sclerotium rolfsi. Sacc. as against 6% when inoculated with the fungus alone. Booth and Stover (1974) found that association of Cylindrocarpon musae with Radopholus similis on bananas was responsible in causing greater damage. Hedrick and Southards (1976) reported an increase in plant mortality when Cylindrocarpon crotolariae was combined with M. incognita and this mortality further increased when nematode inoculation preceded the fungus. Fortnum and Lewis (1978) studied interaction between Cylindrocladium root rot and many nematode populations on soybean and observed that Hoplolaimus columbus and Cylindrocladium crotolariae significantly reduced the population of Pratylenchus scribneri and gall development due to M. incognita. Alcantara et al (1981) observed that culture filtrate of Aspergillus niger, Helminthosporium, Fusarium isolated from body of M. incognita inhibited the activity of Rotylenchulus reniformis after 12 h of contact. Kirmani et al (1980) while attempting to control disease complex reported that soil amendment with different oil cake reduced the severity of disease complex formed by Meloidogyne incognita and Aspergillus versicolor.

c) Nematode fungus seedling disease interactions

Instances of nematode fungus association causing greater damages to seedlings in seedling rot disease are not very uncommon (White, 1962).

Van Gundy and Tsao (1963) reported reduction in growth of citrus seedlings by Fusarium solani as influenced by Tylenchulus semipenetrans. Minton and Minton (1966) observed that sting and root knot nematode adversely affected the emergence of citrus seedlings alone as well as in combination with F. oxysporum. O'Bannon et al (1967) pointed out that growth reduction in rought lemon seedlings was more when T. semipenetrans and Fusarium solani were present together at 30°C. The movement of Pratylenchus penetrans was more towards the roots of alfalfa infected with F. oxysporum (Edmund and Mai, 1967). The emergence of tomato seedlings was found to be adversely affected by M. incognita and Sclerotium rolfsi (Shukla and Swaroop,1970). Whitney (1971) reported a synergistic relationship between Heterodera schachtii and Pythium ultimum and an additive interaction between . P. aphanidermatum and H. schachtii on sugarbeet. Gubina (1975) found an increase in population of Tylenchus ditissimus, Pseudhelenchus and Pratylenchus nannus in presence of Lophoderminum pinastris around the roots of Pinus sylvestris, Adeniji (1975) reported that the seedling rot was more severe in susceptible soybean cultivars, Corsoy and Dyer when H. glycines and Phytophthora megasperma var. sojae (Pms)

were present together than when either of them was present alone. There was however, no such effect on resistant cultivar.Similarly Nbudizu (1977) observed that Meloidogyne hapla or Tylenchorhynchus claytoni alongwith Verticillium dahliae caused more severe growth reduction in cherry seedlings than either the nematode or fungus alone.

Reynolds and Hansen (1957) reported greater severity of post emergence damping-off in cotton caused by Rhizoctonia solani, when M. incognita acrita was present. Norton (1960) pointed out that in the presence of M. incognita acrita the pre-emergence damping off of cotton was more destructive.

Taylor and Wyllie (1959) observed that the interaction of root knot nematode species M. javanica and M. hapla with R. solani caused 87 and 97% seedling mortality respectively in soybean but the percentage of seedling survival increased with the presence of either of the pathogen alone. Similar results were obtained by Polychronopoulas et al (1969,1970). A synergistic interaction between R. solani, P. debaryanum and root knot nematode has been reported in seedling disease complexes of cotton (Brodie 1963). Emergence of cauliflower seedling was adversely affected in the presences of Tylenchorhynchus brassicae and R. solani than in presence of fungus alone (Khan et al 1971).

Carter (1975) reported that synergism between M. incognita and R. solani increased with increase in coarse particle content of soil.

Varshney (1982) studied the interaction of M. incognita and R. solani on cowpea and reported a direct relationship between inoculum potential and reduction in growth, root knot index, and nematode population. He further observed that in sequential inoculations the inoculation of nematode prior to fungus was more effective.

Zakiuddin (1984) observed an increase in reduction of growth of eggplant caused by Rhizoctonia solani in presence of Rotylenchulus reniformis. The reduction in growth was directly proportional to the inoculum potential of both the pathogens and inversely proportional to age of seedlings, however, he observed no significant difference in growth reduction caused by sequential or simultaneous inoculation of both the micro-organisms.

It is evident from the foregoing that most of the interaction studies have been made with two pathogen, one nematode and other fungus under controlled conditions where other micro-organisms were eliminated. This is in contrast to what happens in nature. Therefore, there is a need to study the effect of other non pathogenic microorganisms present in soil (rhizosphere and rhizoplane) on the disease complexes, between nematode and the fungal pathogen.

This may provide some idea of fungi present there which could be used for controlling disease complexes. Moreover, this will also unravel the fact as to why losses due to two pathogens forming disease complexes are more in controlled condition than in nature.

MATERIAL AND METHODS

Chapter - III

MATERIALS AND METHODS

3.1 Raising nursery of test plants

The seedlings of test plant viz. tomato, *Lycopersicum esculentum* Linn. cv. Pusa ruby will be raised in sterilized soil. Until stated otherwise 15-20 days old seedlings will be used for transplantation throughout the course of investigation.

3.2 Pure cultures of *Meloidogyne* sp. and its maintenance

The culture of *Meloidogyne incognita* will be raised by inoculating tomato seedlings from single egg-mass of the nematode taken from infected roots of tomato.

3.3 Inoculation with nematodes

For nematode inoculation second stage larvae will be obtained by allowing the eggmasses to hatch in distilled water. After sufficient amount of larvae have been obtained a suspension with ± 1000 larvae per ml will be prepared. The suspension will be poured near the root zone by making four holes at equidistance around the stem.

3.4 Isolation of fungi from rhizosphere and rhizoplane

For isolating fungi from rhizosphere plants, will be removed carefully from the field and brought to the laboratory in sterilized containers under aseptic

conditions. Blocks of soil adhering to the roots will be removed and crushed gently with minimum damage to the roots. The roots will be shaken gently to remove the superfluous soil. The root pieces thus obtained will be placed in 100 ml of sterilized distilled water and shaken to obtain maximum possible of soil. This will be used as stock solution. Different dilutions upto 1:1000 will be made from stock solution. Now 1 ml of 1:1000 dilution will be placed in sterilized petridishes containing 10 ml of molten, cooled peptone dextrose agar*. The petriplates will be rotated gently in order to obtain equal distribution of soil suspension in the medium.

For each treatment 20 petriplates will be poured. The plates will be incubated at 20°C. Fungi which developing after one week will be examined and identified. The frequency of fungi will be calculated as follows:-

$$\frac{\text{No. of plates containing a particular fungus}}{\text{Total plates poured}} \times 100$$

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* Peptone dextrose agar

KH_2PO_4	-	1.0 g
Peptone		5.0 g
Dextrose		10.0 g
Agar		20.0 g
Distilled water	-	1000 ml
Rose bengal	-	1:30,000 sol.
Streptomycin	-	30 ug/ml

For studying rhizoplane mycoflora wht "serial root washing technique" of Harley and Waid (1955) will be followed. Roots will be washed several times in sterilized distilled water and will be cut into small pieces of 5 mm length and transfered into sterilized petriplates with 5 root pieces in each petriplate containing 10 ml of sterilized cooled, melted peptone dextrose agar medium. Petriplates will be inculated at 28°C.Fungi developing will be examined and identified. The frequency of fungi will be calculated by the method mentioned above.

3.5 Pure culture of fungi and their maintenance

Different fungi isolated from rhizosphere and rhizoplane of tomato seedlings will be made in pure culture by making single spore isolate or hyphal tip isolate (Riker and Riker, 1936). The pure culture will be maintained on sterilized potato dextrose agar (P.D.A.)*. The inoculum of fungus will further be raised from pure culture maintained on P.D.A. in 250 ml Erlenmeyer flaks containing sterilized (15 lbs for 15 mts) Richard's solution of the following constitution.

Potassium nitrate	- 10.0 g
Potassium dihydrogen phosphate	- 5.0 g
Magnesium sulphate	- 2.5 g
Ferric chloride	- 0.02 g
Sucrose	- 50.0 g
Distilled water	- 1000 ml.

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* Potato dextrose agar - 200 g potatoes will be peeled and will be cut into small chips, and boiled in 500 ml of water, the extract will be taken and mixed with 20 g agar and 20 g dextrose boiled already in 500 ml water.

3.6 Inoculum and inoculation with fungus

The mycelial mat of pure culture of the fungus raised above in Richard's solution will be washed in distilled water and blended for 30 seconds in Waring blender for obtaining homogenous suspension. Fungal suspension will be prepared by mixing the mycelium in distilled water at the rate of 10 g of mycelium in 100 ml of sterilized distilled water. Thus each 10 ml of this suspension will contain 1 g of fungus. The seedlings will be inoculated with the desired amount of fungus.

For inoculation, a portion of the soil will be removed from around the roots of test plants and the fungal suspension containing fungus mycelium will be poured and the hole will be covered.

3.7 Recording of observations

3.7.1 Plant growth determination

Plants will be uprooted after 60 days of inoculation. Root system will be thoroughly washed with running water. Length of roots and shoots will be recorded. The excess water of plant will be removed by placing the plant in between the blotting sheets before weighing for fresh weight.

For measuring dry weight the plants will be first kept in oven at 60°C to dry for 2-3 days. After cooling shoots and roots will be weighed seperately.

3.7.2 Root-knot estimation

Intensity of root-knot will be categorised as follows-

Category	No. of galls
1	0 - 50
2	51 - 100
3	101 - 150
4	151 - 200
5	201 - 250

3.7.3 Nematode population estimation

For extraction of nematodes, soil from each treatment will be mixed thoroughly and a sub-sample of 200 g soil will be processed thoroughly through sieve according to Cobb's sifting and gravity method followed by Baermann funnel technique (Southey, 1979).

The suspension will be collected in a beaker and volume made up to 100 ml. For proper distribution of nematodes the suspension will be aerated with the help of pipette and 10 ml suspension will be drawn and transferred to a counting dish. The number of nematodes will be counted. Five such countings will be made. Mean of all such countings will be taken and final population of nematodes/kg of soil will be calculated.

To estimate nematode population of roots, roots from each replicate will be macerated with enough water in

a waring blender for about 10 seconds. The macerate will be collected in a beaker and volume will be made up to 100 ml and nematode population will be counted.

Reproduction factor (R) of the nematode will be calculated by the formula $R = \frac{Pf}{Pi}$ where Pf = Final nematode population, Pi = Initial nematode population.

3.8 Treatments

Unless stated otherwise, the seedlings throughout the course of investigation will be inoculated as under-

a. Inoculation with nematode alone

b. Inoculation with fungus alone

c. Inoculation with nematode + fungus.

3.9 Effect of certain non pathogenic fungi on disease complexes involving root-knot nematode and pathogenic fungus

Pythium aphanidermatum is a serious pathogen on tomato and will be selected for such a study. Species of Aspergillus, Penicillium and Rhizopus from the rhizosphere will be selected for determining their effect on disease complexes.

3.9.1 Effect on seed germination

(a) Seed of different varieties of tomatoes will be germinated in sterilised petridishes containing double layer of blotting. To this will be added 1,2,3, 5 g of suspension of P. aphanidermatum, Aspergillus,

Penicillium, *Rhizopus* and root-knot nematode larvae, seperately or in combination with minimal amount of water sufficient to moist the filter paper. After 2,3,5 and 10 days the number of seeds germinated will be counted and percentage of germination will be calculated. Percentage growth of seeds in sterile water will serve as control.

(b) Seeds will also be germinated in sterile soil infested with above fungi and nematode in different combinations. Percentage growth or emergence of seedlings will be calculated. Percentage emergence in sterile soil will serve as control.

3.9.2 Effect of inoculating seedlings of tomato with non pathogenic fungi on interaction between root-knot nematode and *P. aphanidermatum*

Seedlings of tomato raised above will be inoculated as follows-

1. With nematode
2. With *Pythium aphanidermatum*
3. With *Aspergillus*
4. Nematode + *P. aphanidermatum*
5. Nematode + *Aspergillus*
6. *Aspergillus* + *P. aphanidermatum*
7. Nematode + *Aspergillus* + *P. aphanidermatum*

This will be repeated with Penicillium and Rhizopus in the above manner.

These tests will be made with the seedlings obtained in 'b' above and showing minimal growth. Uninoculated plants will be kept for control.

3.10 Effect of culture filtrate on hatching

Culture filtrate of different fungi will be obtained by growing them in Richard's solution. After growing for 15 days, the contents of the flasks will be filtered through whatman filter paper. The filtrate will be termed as standard 'S' and will be diluted to 0.1, 0.01, 0.001, with sterile distilled water.

Ten ml of the culture filtrate of different fungi, will be poured in petridishes to which 20 freshly collected eggmasses will be added. The filtrate of different fungi will be tested separately and in combination. The plates will be incubated at 20°C.

After 2, 3, 5, 7 days the number of larvae hatched out will be counted and cumulated hatch will be determined. Hatching in distilled water will serve as control.

3.11 Effect of different carbon and nitrogen sources on the growth of the fungus exhibiting highest inhibitions

The fungus exhibiting highest inhibition will be tested for the effect of the different carbon and

nitrogen sources so as to obtain highest inhibiting culture filtrate. The carbon sources tested will be as follows.

1) Glucose
2) Sucrose
3) Fructose
4) Arabinose
5) Xylose
6) Ribose
7) Rhamnose
8) Starch

Nitrogen sources will be

1) Potassium nitrate
2) Ammonium nitrate
3) Ammonium chloride
4) Urea
5) Asparagnine
6) Glycine
7) Lysine
8) Methionine.

The Richard's solution containing sucrose and potassium nitrate will be used as basal medium. The potassium nitrate will be replaced by the above nitrogen sources and sucrose by the above carbon sources so as to keep C or N level constant. The fungus will be grown in these for 15 days. The culture filtrate so obtained will be tested for hatching of larvae in the manner

Throughout the studies the number of replicates will be kept constant. The data will be analysed statistically.

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